PROJECT NUMBER: 6912
PROJECT TITLE: Tobacco/Smoke Relationships
PROJECT LEADER: S. B. Hassam
PERIOD COVERED: April, 1989

I. TSNA PRECURSORS

A. **Objective**: To determine the precursors of MS TSNA.

B. **Results**: Radiolabeled nicotine cigarettes (code 3-89-3) were smoked on a single port total recovery smoking machine by Dick Newman of the Chemical Research Division (CRD). Two smokings of five cigarettes each were done (4/5/89, 4/12/89). In each set, MS TPM and SS TPM were collected from each cigarette on disposable Cambridge pads. Fresh pads were used for each cigarette. The TPM pads were shaken in citrate/phosphate buffer containing L-ascorbic acid. From each smoking the MS TPM buffer extract was extracted with methylene chloride. The organic phase was washed with 2N sodium hydroxide, dried over sodium sulphate, and excess solvent was removed under reduced pressure. The residue was redissolved in methylene chloride (10 mL).

Liquid scintillation counting (LSC) of the buffer extracts from smoking #1 yielded the following results: MS TPM buffer extract, 6.44 µCi; SS TPM buffer extract, 10.51 µCi; SS buffer rinse of smoking chamber, 4.74 µCi. The results for smoking #2 were as follows: MS TPM buffer extract, 6.36 µCi; SS TPM buffer extract, 9.90 µCi; SS buffer rinse of chamber, 5.89 µCi. (The estimated initial activity per 5 cigarettes is about 50 µCi, based on values determined by sectioning 2 cigarettes; the radioactivities in the gas phases and in the butts were determined by Dick Newman and the total recovered activity is estimated to be about 90%).

Radioactivities for the methylene chloride solutions obtained from the MS TPM buffer extracts were: smoking #1, 233 nCi; smoking #2, 257 nCi. Preliminary radiochromatography on TLC plates of the MS TPM buffer extracts and methylene chloride solutions indicated that virtually all of the radioactivity is associated with ^{14}C-nicotine.

C. **Plans**: Continue radiochromatography of MS TPM extracts. Isolate TSNA by preparative TLC and analyze by radiochromatography and by GC/TEA. Nonradiolabeled cigarettes of the same filler blend and configuration obtained from CRD will be smoked and analyzed for TSNA by Project 6908.

D. **Reference**:

Hassam, S. Notebook No. 8712, p. 179.

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II. CROSSED SOLUBLES/BASE WEB STUDY (CHEMISTRY)

A. **Objective**: To investigate the smoke chemistry of model cigarettes made from all possible combinations of solubles from bright, burley and oriental tobaccos on base webs from the three tobaccos.

B. **Results**: The preparation of fillers using bright base web and burley CEL, each containing different levels of added calcium acetate, was completed. Handmade cigarettes were prepared from each of the 23 filler types. Cigarettes were smoked in quadruplicate, 5 cigarettes per smoking to provide IT CSC. The CSC from each smoking was dissolved in DMSO for S/M assay.

The preparation of fillers using bright base web and bright CEL containing different levels of our standard protein was completed. Fillers were also prepared with bright CEL containing different amounts of an amino acid mixture, and different amounts of ammonium acetate. The preparation of the fillers was done in a collaborative effort with E. Lambert (Project 6908).

Elution of a weakly acidic cation exchange resin previously treated with burley CEL was continued with 0.05M ammonium acetate. This treatment yielded 7.1 g of additional material. A final treatment with 0.05M sodium hydroxide yielded 1.8 g of material. The total recovery of material, including 51.4 g eluted initially with water, and 15.5 g eluted with methanol and methanol/acetic acid, was 46.7% of the burley CEL treated with the resin.

Burley CEL (171.8 g) was treated with an ion retardation resin (Bio-Rad AG 11A8, pH 9.5) in water. The resin was removed by filtration and then stirred in water. The aqueous filtrates (pH 7.3) were combined and concentrated on a rotary evaporator and then dried *in vacuo*, to yield 64.6 g of material. To remove material bound to the resin, the resin was washed twice with 0.5M ammonium acetate to yield 31.5 g of material, and then with 0.05M acetic acid to yield 9 g of material. The total recovery was 61%.

Burley CEL (190.8 g) was treated with a chelating ion exchange resin (Bio-Rad Chelex 100, pH 5.9 after treatment with sodium acetate buffer, followed by washing with water). Concentration of the resulting aqueous filtrate (pH 5.2) is in progress.

C. **Plans**: Preparation of fillers and cigarettes will be continued. Cigarettes will be smoked for S/M assay. Samples from the ion exchange treatments will be prepared for spraying. Overhauling and servicing of the electrodialysis unit will be continued. Formulation of plans for C Pilot Plant production of RL will be continued.

D. **References**:

Hellams, R. Notebook No. 8613, pp. 153-154.
McGee, N. Notebook No. 8743, p. 40.
Drew, S. Notebook No. 8800, pp. 22-23.
Hassam, S. Notebook No. 8712, p. 179.

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III. OPTIMIZATION OF A LOW ACTIVITY MODEL: MS NITRIC OXIDE CONTENT

A. **Objective**: To develop methods of reducing MS NO delivery for a low activity model.

B. **Results**: Two model cigarettes containing an NO oxidant and an NO_2 absorbent were prepared using a dual plug/space/plug filter configuration and all burley filler. The oxidant was a catalyst containing chromium trioxide and potassium dichromate on silica gel. The absorbent was triethanolamine on florisil. The models contained different ratios of oxidant/absorbent. Using current methodology, NO deliveries were determined for these cigarettes. No significant reduction was observed with these cigarettes.

C. **Plans**: The use of zeolites as potential catalysts for NO decomposition will be investigated.

D. **Reference**:

Levins, R. J. Notebook No. 8757, p. 172.

IV. DEVELOPMENT AND APPLICATION OF ANALYTICAL PROCEDURES

A. **Objective**: To develop, maintain and apply analytical methodology for minor alkaloids and other compound classes.

B. **Results**: Twenty-two samples of tobacco filler and eleven ethanol extracts of bright filler obtained from a multiple pass system were analyzed for minor alkaloids and nicotine by capillary GC/NPD.

C. **Reference**:

Levins, R. J. Notebook No. 8757, p. 171.

V. SUPPORT FUNCTION: CONDENSATE PREPARATION

A. **Objective**: To fabricate cigarettes, perform smokings, and prepare condensate as needed for biological and chemical analysis.

B. **Results**: Three types of fillers were prepared for project 6908: bright base web was sprayed with bright CEL and bright CEL mixed with DAP; burley base web was sprayed with a mixture of bright CEL and burley CEL. Cigarettes were prepared and smoked for other BCR staff. Sixty cigarettes each of four cigarette models were smoked to provide condensate in special plastic containers, for Bob Jenkins (CRD).

C. **References**:

Hellams, R. Notebook No. 8613, p. 153-154.
McGee, N. Notebook No. 8743, p. 40.

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